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The Fate of Certain Organic Acids and Amides in the Rabbit

12. AMINOHYDROXYBENZOIC ACIDS

By H. G. BRAY, W. V. THORPE and P. B. WOOD
Physiology Department, Medical School, University of Birmingham

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Our interest in the aminohydroxybenzoic acids arose from the finding that these acids or their amides were excreted after the administration to rabbits of the aminobenzamides (Bray, Lake, Neale, Thorpe & Wood, 1948) and *o*-nitrobenzamide (Bray, Thorpe & Wood, 1949). 2-Amino-3-hydroxy- and 2-amino-5-hydroxy-benzoic acids were isolated from hydrolysed *o*-aminobenzamide urine, 3-amino-6-hydroxybenzoic acid (5-amino-2-hydroxybenzoic acid) from hydrolysed *m*-aminobenzamide urine, 4-amino-3-hydroxybenzoic acid from hydrolysed *p*-aminobenzamide urine and 2-amino-5-hydroxybenzoic acid from hydrolysed *o*-nitrobenzamide urine. It was also suspected that 3-amino-4-hydroxybenzoic acid (or its amide) was present in *m*-aminobenzamide urine and 2-amino-3-hydroxybenzoic acid (or its amide) in *o*-nitrobenzamide urine, although these were not isolated. It was, however, shown later, using paper chromatography, that all the theoretical products of ortho and para hydroxylation are present in aminobenzamide and *o*-nitrobenzamide urines (Bray, Lake, Thorpe & White, 1950).

Two aminohydroxybenzoic acids have recently received attention by other workers. 4-Amino-2-hydroxybenzoic acid ('*p*-aminosalicylic acid', 'PAS') has been used in the treatment of tuberculous lesions in human patients (e.g. Lehmann, 1946; Dempsey & Logg, 1947), and some investigations of its metabolism have been reported (e.g. McClosky, Smith & Frias, 1948; Way, Weiss, Howie & Smith, 1948). In a more detailed study Venkataraman, Venkataraman & Lewis (1948) concluded that 40–60 % of the administered acid was excreted by the rabbit in 24 hr. as the *N*-acetyl derivative. No decarboxylation, glycine conjugation or *O*-conjugation was observed.

2-Amino-3-hydroxybenzoic acid has been shown to be a precursor of nicotinic acid in *Neurospora* (Mitchell & Nyc, 1948; Bonner, 1948) and in the rat (Mitchell, Nyc & Owen, 1948; Heidelberger, Abraham & Lepkovsky, 1948, 1949; Albert, Scheer & Deuel, 1948). It is suggested that this acid may be an intermediate in the conversion of tryptophan into nicotinic acid. No investigations of the metabolic fate of large doses of 2-amino-3-hydroxybenzoic acid appear to have been carried out, though Henderson & Hirsch (1949) reported that rats which had received intraperitoneal injections of this acid excreted quinolinic acid (pyridine-2:3-dicarboxylic acid).

In the present investigation, which is an extension of that previously reported in brief (Bray, Ryman & Thorpe, 1948*b*), we have studied the fate of seven of the ten aminohydroxybenzoic acids and have determined the main metabolic pathways of six of them. 2-Amino-4-hydroxy and 3-amino-5-hydroxybenzoic acids have been prepared, but in yields so small as to render accumulation of the acids for metabolic investigation impracticable. Attempts to prepare 2-amino-6-hydroxybenzoic acid were unsuccessful.

MATERIALS

2-Amino-3-hydroxybenzoic acid was prepared as previously described (Bray, Lake, Neale, Thorpe & Wood, 1948).

2-Amino-4-hydroxybenzoic acid. Small amounts of this acid, m.p. 158° (decomp.), were prepared by a method used by Prof. F. S. Spring and made available to us by Mr D. E. Seymour (Herts Pharmaceuticals Ltd.). Yields were, however, very small. The acid is very readily decarboxylated to *m*-aminophenol by heating in aqueous solution particularly at acid pH.

2-Amino-5-hydroxybenzoic acid was prepared as previously described (Bray, Lake, Neale, Thorpe & Wood, 1948).

2-Amino-6-hydroxybenzoic acid. 2-Amino-6-nitrobenzoic acid was prepared by Hofmann degradation of 3-nitrophthalamic acid, but attempts to obtain 2-amino-6-hydroxybenzoic acid from 2-amino-6-nitrobenzoic acid by a procedure similar to that described below for 3-amino-5-nitrobenzoic acid were unsuccessful. (2-Amino-6-nitrobenzoic acid is readily decarboxylated in acid solution.)

3-Amino-2-hydroxybenzoic acid was obtained by reduction of 3-nitrosalicylic acid (British Drug Houses Ltd.) with $SnCl_2$ and HCl (cf. Zahn, 1900).

3-Amino-4-hydroxybenzoic acid was prepared by hydrolysis of its methyl ester, orthocaine (British Drug Houses Ltd.).

3-Amino-5-hydroxybenzoic acid (with Dr F. C. Neale). A solution of 3:5-dinitrobenzoic acid (20 g. in 250 ml. 96% (v/v) ethanol) was saturated with H_2S at ordinary temperature. After addition of NH_3 (100 ml., sp.gr. 0·880) H_2S was passed for a further 30 min. (More prolonged treatment with H_2S gives 3:5-diaminobenzoic acid.) The solution was evaporated to dryness and the residue, crystallized from aqueous ethanol, gave orange needles of 3-amino-5-nitrobenzoic acid, m.p. 209–210°. (Found: N, 15·3. Calc. for $C_7H_6O_4N_2$: N, 15·4%.) Yield 10 g. Treatment of this compound with acetic anhydride gave *3-acetamido-5-nitrobenzoic acid*, which on recrystallization from 96% ethanol formed a dull yellow powder, m.p. 283° (decomp.). (Found: N, 12·9%. $C_9H_8O_5N_2$ requires N, 12·5%.) Yield 10 g. from 10 g. 3-amino-5-nitrobenzoic acid. Reduction with $FeSO_4$ and NH_3 (cf. Jacobs & Heidelberger, 1917) gave light-yellow needles of *3-acetamido-5-aminobenzoic acid monohydrate*, m.p. 230°. (Found: C, 51·3; H, 5·7; N, 13·0. $C_9H_{12}O_4N_2$ requires, C, 51·0; H, 5·7; N, 13·2%.) Yield 1 g. from 10 g. 3-acetamido-5-nitrobenzoic acid. Treatment with $NaNO_2$ and HCl followed by decomposition of the diazo compound by boiling gave *3-amino-5-hydroxybenzoic acid* as colourless prisms, m.p. 242°. (Found: C, 54·2; H, 4·6; N, 9·3. $C_7H_7O_3N$ requires C, 54·9; H, 4·7; N, 9·2%.) Yield 0·5 g. from 2·5 g. 3-acetamido-5-aminobenzoic acid. The constitution of the compound was confirmed by conversion into 3:5-dihydroxybenzoic acid by diazotizing and boiling. The dihydroxy acid had m.p. 232° alone or mixed with an authentic specimen prepared by alkali fusion of the Ca salt of 3:5-disulphobenzoic acid (Barth & Senhofer, 1871). (Found: C, 54·7; H, 4·0. Calc. for $C_7H_6O_4$: C, 54·6; H, 3·9%.) The identity of the two dihydroxy compounds was also confirmed on paper chromatograms. Occasionally the product obtained by reduction of 3-acetamido-5-nitrobenzoic acid consisted of yellow needles, m.p. 265° instead of 230°. This compound, which also gave 3-amino-5-hydroxybenzoic acid on diazotizing and boiling, was *3-acetamido-5-aminobenzoic acid sulphate dihydrate.* (Found: C, 41·6; H, 5·0; N, 10·3. $C_{18}H_{26}O_{12}N_4S$ requires C, 41·4; H, 5·0; N, 10·7%.)

3-Amino-6-hydroxybenzoic acid was prepared by reduction of 5-nitrosalicylic acid (British Drug Houses Ltd.) with $SnCl_2$ and HCl (cf. Zahn, 1900).

4-Amino-2-hydroxybenzoic acid. We are indebted to Mr D. E. Seymour for the gift of this acid and its Na salt.

4-Amino-3-hydroxybenzoic acid was prepared as previously described (Bray, Lake, Neale, Thorpe & Wood, 1948).

Preparation of acetamidohydroxybenzoic acids. These were obtained by treatment of the aminohydroxybenzoic acids with acetic anhydride at ordinary temperature. 3-Amino-6-hydroxybenzoic acid forms the *ON*-diacetyl derivative so readily that preparation of 3-acetamido-6-hydroxybenzoic acid is difficult, although this compound is easily obtained from 3-amino-6-hydroxybenzoic acid urine.

METHODS

Diet and dosage. The rabbits used were does weighing 2–3 kg. maintained throughout the investigation on the constant diet of rabbit pellets and water used in this laboratory (Bray, Ryman & Thorpe, 1947). The acids (0·6–1·0 g./rabbit) were administered by stomach tube as suspensions in water, without previous neutralization. No toxic effects were observed with any of the compounds.

Estimation of aminohydroxybenzoic acids. The modified Ehrlich reaction described by Venkataraman *et al.* (1948) was used for the determination of compounds possessing a free amino group. The method is less sensitive than a diazotization method, but was used because of its general applicability since several of the acids studied did not diazotize and couple normally. Satisfactory results were obtained if the standard solutions used were of approximately the same strength as the unknowns. The method was applicable to all the aminohydroxybenzoic acids except 2-amino-3-hydroxybenzoic acid, which gave only a very feeble colour with the reagent. No satisfactory method was found for the colorimetric estimation of this acid. Determinations were made on urines or extracts before and after hydrolysis. For hydrolysis, urine or solution was heated with HCl (2 ml. 2N/5 ml.) for 1 hr. in a boiling-water bath. Hydrolysed solutions were treated with NaOH (2 ml. 2N) before development of the colour. Control experiments showed that the hydrolysis procedure caused decarboxylation of only 4-amino-2-hydroxybenzoic acid. (*m*-Aminophenol was detected by paper chromatography.) The intensity of colour given by *m*-aminophenol with the Ehrlich reagent was 94% of that given by an equimolecular amount of 4-amino-2-hydroxybenzoic acid.

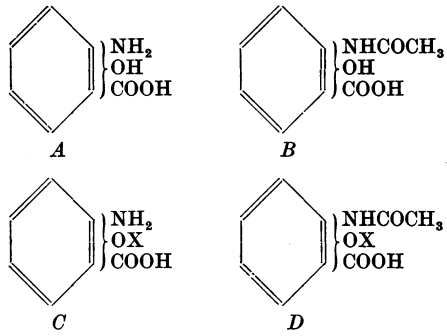
Estimation of ethereal sulphate. Folin's method (1905–6) was used.

Estimation of ether-soluble acid. Urine (20 ml.) was continuously extracted with ether at a pH and for a period of time found in control experiments to give complete recovery of the aminohydroxybenzoic acid added to normal rabbit urine. Paper chromatography of such extracts of metabolic urines and of similar extracts made subsequently below pH 1 showed that this procedure resulted also in the complete extraction of the corresponding acetamidohydroxybenzoic acids. Extraction at pH 4 was suitable for all the acids studied except 4-amino-2-hydroxybenzoic acid, for which pH 3·1 was necessary. The ether-soluble material was titrated to pH 8 (pH 7·4 for 4-amino-2-hydroxybenzoic acid) with NaOH (0·05N). Both free and acetylated acids are titrated at this pH.

Paper chromatography. The technique was essentially that previously described (Bray, Thorpe & White, 1950).

Reducing material. This was not estimated since the aminohydroxybenzoic acids reduce alkaline cupric salts.

Calculation of results. Preliminary experiments indicated that the aminohydroxybenzoic acids might be excreted in four ways: (1) unconjugated, (*A*); (2) conjugated through the amino group only, (*B*); (3) conjugated through the hydroxyl group only, (*C*); (4) conjugated through both amino and hydroxyl groups, (*D*).



It was assumed, no evidence to the contrary being obtained, that *C* and *D* are not extracted from urine by ether. Determinations by the Ehrlich method should provide the following information:

On urine as collected	$A+C$
On hydrolysed urine	$A+B+C+D$
On material extracted by ether	A
On material extracted by ether and then hydrolysed	$A+B$
On urine left after ether extraction	C
On the above after hydrolysis	$C+D$

Determinations, however, on urines left after ether extraction tended to give low results and were abandoned after preliminary experiments. The remaining determinations provided sufficient data for calculation of values for *A*, *B*, *C* and *D*. Estimation of ether-soluble acid also gave $A+B$. Paper chromatography of the ether extracts also gave approximate values for *A* and *B*.

RESULTS

Normal excretion of metabolites

The average daily excretion of ethereal sulphate by the rabbits used in this investigation ranged from 25 to 54 mg. SO_3. The average percentage by which the normal individual daily values differed from the corresponding weekly averages used as 'baseline' for purposes of calculation was $\pm 8\%$ (± 4 mg. SO_3). The corresponding values for ether-soluble acid were 470–815 mg. (calculated as hippuric acid) and $\pm 5\%$ for extractions at pH 4·0 and 748–833 mg. and $\pm 2\%$ for extractions at pH 3·1. The Ehrlich reagent gave a slight yellow colour with normal rabbit urine. This corresponded to an average daily excretion of 54 mg. calculated as aminohydroxybenzoic acid. The material responsible for this colour was not extracted by ether, or affected by hydrolysis to an appreciable extent.

Metabolites of aminohydroxybenzoic acids

The results of quantitative analysis of urines of rabbits after administration of aminohydroxybenzoic acids are given in Table 1. The results by the Ehrlich method have been calculated on the assumption that the *O*-conjugates give the same intensity of colour with the reagent as the free acid. Since neither glucuronides nor ethereal sulphates of the acids have been prepared the validity of this assumption could not be proved. Since, however, the $(A+B)$ results, which are in reasonable agreement with those obtained by determination of ether-soluble acid, account for at least two-thirds of the acid administered, errors due to differing colour intensities from *O*-conjugates $(C+D)$ cannot affect more than one-third of the total amount of acid recovered. Unless, therefore, the difference in colour intensities is very pronounced, errors due to this will cause relatively little distortion of the general

Table 1. *Excretion of aminohydroxybenzoic acids in the rabbit*

(The results are expressed as average percentage of dose; ranges in parentheses. Superior figures against ranges indicate the number of experiments. Dose 1 g. throughout, except 0·6 g. for 2-amino-3-hydroxybenzoic acid.)

	Percentage of dose estimated by Ehrlich reagent in				Excretion as	
Compound	Urine as collected ($A+C$)	Urine after hydrolysis ($A+B+C+D$)	Ether extract of urine (A)	Hydrolysed ether extract ($A+B$)	Ether-soluble acid ($A+B$)	Ethereal sulphate
2-Amino-3-hydroxybenzoic acid*	—	—	—	—	—†	3 (2–4)[3]
2-Amino-5-hydroxybenzoic acid	75 (64–95)[10]	101 (90–112)[4]	71 (65–77)[4]	85 (80–91)[4]	95 (77–114)[8]	2 (0–4)[4]
3-Amino-2-hydroxybenzoic acid	42 (40–44)[2]	95 (94–95)[2]	35 (34–35)[2]	76 (74–78)[2]	80 (70–96)[8]	0[5]
3-Amino-4-hydroxybenzoic acid	58 (40–75)[18]	75 (64–82)[9]	44 (37–50)[5]	64 (58–68)[4]	60 (40–75)[19]	7 (4–16)[6]
3-Amino-6-hydroxybenzoic acid	46 (18–76)[6]	93 (77–113)[6]	28 (10–51)[6]	62 (34–81)[6]	67 (33–97)[6]	2 (0–4)[4]
4-Amino-2-hydroxybenzoic acid‡	32 (12–45)[11]	72 (62–77)[5]	31 (15–49)[5]	62 (60–68)[5]	66 (53–76)[6]	0[5]
4-Amino-3-hydroxybenzoic acid	34 (23–45)[8]	107 (104–112)[3]	22 (20–25)[3]	84 (77–89)[3]	54 (43–55)[5]	8 (5–9)[3]

* Ehrlich values could not be determined for this acid. See section on estimation of aminohydroxy acids, p. 395.

† Values were too small to be distinguished with certainty from baseline values.

‡ This acid undergoes partial decarboxylation both *in vivo* and during hydrolysis of the urine.

Table 2. *Metabolites excreted by rabbits after administration of aminohydroxybenzoic acids*

(The results are mainly calculated from data of Table 1.)

Compound	Average percentage of dose excreted as: Unchanged acid *A*	*N*-Acetamido-hydroxy acid *B*	*O*-Conjugated amino-acid *C*	*O*-Conjugated acetamido-acid *D*	Ethereal sulphate	Total percentage of dose accounted for
2-Amino-3-hydroxybenzoic acid*	10†	0†	3†	0†	3	13
2-Amino-5-hydroxybenzoic acid‡	71	14	4	12	2	101
3-Amino-2-hydroxybenzoic acid*	35	41	7	12	0	95
3-Amino-4-hydroxybenzoic acid*‡	44	20	14	0	7	78
3-Amino-6-hydroxybenzoic acid	28	34	18	13	2	93
4-Amino-2-hydroxybenzoic acid‡	31	31	1	9	0	72
4-Amino-3-hydroxybenzoic acid*	22	62	12	11	8	107

* Positive diazo reactions were given by these urines. This confirms *O*-conjugation in column *C* since the acids administered do not couple after diazotization owing to formation of diazo oxides.

† See text for basis upon which these values were calculated.

‡ Determination of glucuronide showed that the *O*-conjugates in the urines could be accounted for as glucuronide and ethereal sulphate.

picture. For three acids (see Table 2) the results of estimations of glucuronide in the urines by a modification of the naphthoresorcinol method of Hanson, Mills & Williams (1944) approximated to the value for ($C+D$-ethereal sulphate), so that at least for these acids errors due to different colour intensities appear to have little significance. Where more experiments are recorded in the ($A+C$) column of Table 1 than for the other columns giving results of Ehrlich determinations, the average values obtained from all experiments were not significantly different from the average ($A+C$) for those experiments in which all the Ehrlich values were determined.

The values recorded in Table 2 have been calculated mainly from the average results given in Table 1. The greater part of the dose has been accounted for for all acids except 2-amino-3-hydroxybenzoic acid, for which the Ehrlich method could not be used. The *A* and *B* values recorded for this acid are only approximate values found by means of paper chromatograms (Table 4). 2-Amino-3-hydroxybenzoic acid urine gave a feeble diazo reaction, which was presumably due to an *O*-conjugate, in which the amino group was free, since the acid itself gave no diazo reaction. The intensity of the colour was such as might have been expected if an amount of conjugate corresponding to that found as ethereal sulphate had been present. Examination of the urine for glucuronide revealed no increase above the normal excretion. For these reasons the *C* value recorded in Table 2 is 3%, the value found for ethereal sulphate, and the *D* value is given as 0. The absence of all conjugates except small amounts of ethereal sulphate from the urine is supported by the fact that, when urine which had been exhaustively extracted with ether was hydrolysed and then again extracted with ether, the second extract showed only a barely perceptible fluorescence. Free 2-amino-3-hydroxybenzoic acid gives a pronounced mauve fluorescence in ethereal solution.

Paper chromatography of ether extracts of aminohydroxybenzoic acid urines

Ether extracts of the metabolic urines at pH 4·0 were examined by paper chromatography as previously described (Bray, Thorpe & White, 1950). The solvent mixtures used and the R_F values of the compounds are given in Table 3. The detecting reagent used was diazotized *p*-nitraniline. By comparison of the size and intensity of the spots produced by running the ether extracts alongside standard solutions rough estimates (within ± 20%) of the amounts of free and acetylated aminohydroxybenzoic acids present (*A* and *B*) were made. The results, which are given in Table 4, provide, within the limits of experimental error, reasonable confirmation of the corresponding values in Table 2.

Qualitative examination of aminohydroxybenzoic acid urines

The ether-soluble material obtained by continuous extraction of aminohydroxybenzoic acid urines at pH 4 (pH 3·1 for 4-amino-2-hydroxybenzoic acid) was purified by recrystallization from water. 3-Amino-2-hydroxy-, 3-amino-4-hydroxy-, 3-amino-6-hydroxy- and 4-amino-3-hydroxy-benzoic acid urines yielded both the free acids and the acetamido compounds which were identified by comparison with authentic specimens. 2-Amino-3-hydroxy- and 2-amino-5-hydroxy-benzoic acid urines yielded only the free acids. Only the *N*-acetyl derivative was isolated from 4-amino-2-hydroxybenzoic acid urine, although the presence of the unacetylated acid was shown by paper chromato-

Table 3. *R_F Values of some aminohydroxybenzoic acids and their* N*-acetyl derivatives*

(Solvent mixtures: A, chloroform (2 vol.), acetic acid (2 vol.), water (1 vol.); B, benzene (2 vol.), acetic acid (2 vol.), water (1 vol.); C, *n*-butanol (4 vol.), pyridine (8 vol.), saturated aqueous NaCl (5 vol.), ammonia (sp.gr. 0·880, 3 vol.); D, *iso*propanol (4 vol.), pyridine (8 vol.), saturated aqueous NaCl (5 vol.), ammonia (sp.gr. 0·880, 3 vol.). Paper, Whatman no. 4.)

Compound	Solvent mixture	Time of run (hr.)	R_F value
2-Amino-3-hydroxybenzoic acid	B	4	0·3
2-Acetamido-3-hydroxybenzoic acid	B	4	0·9
2-Amino-5-hydroxybenzoic acid	A	6	0·18
2-Acetamido-5-hydroxybenzoic acid	A	6	0·75
3-Amino-2-hydroxybenzoic acid	A	6	0·48
	B	8	0·20
3-Acetamido-2-hydroxybenzoic acid	A	6	1·0
	B	8	0·67
3-Amino-4-hydroxybenzoic acid	A	6	0·24
3-Acetamido-4-hydroxybenzoic acid	A	6	0·80
3-Amino-5-hydroxybenzoic acid	A	6	0·20
3-Amino-6-hydroxybenzoic acid	C	3	0·57
	D	5	0·8
3-Acetamido-6-hydroxybenzoic acid	C	3	0·54
	D	5	0·8
4-Amino-2-hydroxybenzoic acid	A	6	0·91
	B	6	0·87
4-Acetamido-2-hydroxybenzoic acid	A	6	0·91
	B	6	0·33
4-Amino-3-hydroxybenzoic acid	A	6	0·60
4-Acetamido-3-hydroxybenzoic acid	A	6	0·87

Table 4. *Excretion of amino- and acetamido-hydroxybenzoic acids as estimated by paper chromatography of ether extracts of urine*

(No solvent mixture was found which separated 3-amino- and 3-acetamido-6-hydroxybenzoic acids.)

Compound administered	No. of experiments	Approximate percentage of dose: Unacetylated (*A*) Range	Unacetylated (*A*) Average	Acetylated (*B*) Range	Acetylated (*B*) Average
2-Amino-3-hydroxybenzoic acid	6	8–12	10	0	0
2-Amino-5-hydroxybenzoic acid	4	41–62	51	21–41	31
3-Amino-2-hydroxybenzoic acid	3	40–65	55	35–60	46
3-Amino-4-hydroxybenzoic acid	3	All 50	50	40–50	43
4-Amino-2-hydroxybenzoic acid	5	8–25	17	42–75	60
4-Amino-3-hydroxybenzoic acid	3	21–24	22	62–76	69

graphy. Of the seven aminohydroxybenzoic acids studied only 4-amino-2-hydroxybenzoic was shown to be decarboxylated by the hydrolysis procedure used in the quantitative studies (p. 395). It also appears to be decarboxylated to a small extent *in vivo*, since *m*-aminophenol was detected by paper chromatography in the extracts obtained when 4-amino-2-hydroxybenzoic acid urine was extracted with ether at pH 6. The results in Table 2 indicate the formation of appreciable amounts of *O*-conjugates from all except 2-amino-3-hydroxybenzoic acid, but attempts to isolate glucuronides from the urines after administration of 3-amino-4-hydroxy-, 3-amino-6-hydroxy- and 4-amino-3-hydroxy-benzoic acids by the usual lead procedure (Bray *et al.* 1947) were unsuccessful. The corresponding acetamido compounds were the only compounds isolated.

DISCUSSION

Theoretically there are three conjugable groups in aminohydroxybenzoic acids. It appears, however, from the results given in Tables 1 and 2 that only the amino and the hydroxyl groups are conjugated in the rabbit. *N*-Acetylation occurs with all the isomers studied except 2-amino-3-hydroxybenzoic acid. All except 2-amino-3-hydroxy- and 3-amino-4-hydroxy-benzoic acids appear to be excreted with

both hydroxyl and amino groups in the same molecule conjugated. The introduction of an amino group into salicylic acid does not facilitate sulphate conjugation (see Bray, Ryman & Thorpe, 1948*a*). None of the aminohydroxybenzoic acids studied is conjugated with sulphate to a great extent.

2-Amino-3-hydroxybenzoic acid was the only one of the seven acids examined which could not be satisfactorily accounted for by the methods used in this investigation. It may be pertinent that this acid, as already mentioned, has been suggested as an intermediate in the biological conversion of tryptophan into nicotinic acid. The view that quinolinic acid may be an intermediate in the conversion of 2-amino-3-hydroxybenzoic acid into nicotinic acid is supported by the observations of Henderson & Hirsch (1949) that, in addition to small amounts of nicotinic acid, the urine of rats which had been given 2-amino-3-hydroxybenzoic acid (153 mg.) intraperitoneally contained amounts of quinolinic acid corresponding to 16% of the dose. In rabbit urine we could only account for about 13% of a dose of 2-amino-3-hydroxybenzoic acid (600 mg. by stomach tube) as the unchanged acid together with a small amount of ethereal sulphate. If the rabbit had excreted amounts of quinolinic acid comparable to those found by Henderson & Hirsch for the rat this should have been detected in the ether-soluble acid material.

French & Freedlander (1949) have suggested that the tuberculostatic action of 4-amino-2-hydroxybenzoic acid may be due to the metabolic formation of an aminodihydroxybenzoic acid. We obtained no evidence for the presence of such a metabolite in urine. From our previous experience (see Thorpe, 1950) we should not expect hydroxylation to occur to any appreciable extent in a compound which is itself readily excreted and in which two of the three conjugable groups are readily conjugated.

SUMMARY

1. The fate of seven aminohydroxybenzoic acids in the rabbit has been investigated.

2. All the acids are excreted unconjugated to degrees varying from 10 to 71% of the dose, and all except 2-amino-3-hydroxybenzoic acid as *N*-acetyl derivatives (14–62% of the dose).

3. All the acids are excreted partly as *O*-conjugates either unacetylated or acetylated.

4. All except 3-amino-2-hydroxy- and 4-amino-2-hydroxy-benzoic acids are excreted conjugated to a small extent with sulphuric acid (2–8% of the dose).

5. No conjugation of the carboxyl group was detected.

6. A small amount of decarboxylation of 4-amino-2-hydroxybenzoic acid was detected.

7. Only 13% of a dose of 2-amino-3-hydroxybenzoic acid could be accounted for.

8. 3-Amino-5-hydroxybenzoic acid has been synthesized.

9. R_F values for amino- and acetamido-hydroxybenzoic acids are recorded.

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